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EXAMINER	
WOOLWINE, SAMUEL C	

ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/562,840	DRESSMAN ET AL.	
	Examiner	Art Unit	
	SAMUEL C. WOOLWINE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *17 March 2010*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *37,39,43-45,60,62 and 85-90* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☒ Claim(s) *37,39,43,60 and 62* is/are allowed.

6)☒ Claim(s) *44,45 and 85-90* is/are rejected.

7)☒ Claim(s) *44* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *02/03/2010;05/18/2010*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status

Applicant's reply filed 03/17/2010 is acknowledged. Claims 37, 39, 43-45, 60, 62 and 85-90 are pending (claims 85-90 are new).

The rejection of claims 37, 39, 60 and 62 under 35 USC 112, 2nd paragraph made in the Office action mailed 12/28/2009 is withdrawn in view of Applicant's amendments to those claims.

The rejection of claim 44 under 35 USC 102(e) over Leamon et al (US 7,323,305) is withdrawn in view of Applicant's arguments which have been found persuasive: the mere collection of data is not inherently a particular analysis of the data.

New grounds of rejection are set forth for claims 44, 45 and new claims 85-90. Claim 45 is rejected as necessitated by amendment. Claims 44 and new claims 85-90 are rejected based in part on a reference cited on the IDS submitted 02/03/2010 (Vogelstein et al, 1999). However, as this reference was previously considered on the IDS submitted 07/05/2007, the rejection will not be made final based on its re-submission on the latest IDS.

This action is NON-FINAL.

Claim Objections

Claim 44 is objected to because of the following informalities: in the last step, "determining amount of product beads..." appears to be missing "an" or "the" between the word "determining" and the word "amount". Appropriate correction is required.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 45 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

"The method of claim 44 wherein the are determined using flow cytometry" does not make any sense. Presumably this should read "wherein the amount is determined by flow cytometry".

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 44 and 85-90 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, prior art of record) in view of Vogelstein et al (PNAS 96:9236-9241, August 1999, cited on the IDS of 02/03/2010).

With regard to claims 44 and 85, Leamon taught:

forming microemulsions comprising one or more species of analyte DNA molecules

See Leamon claim 1, step b: "delivering the fragmented nucleic acids into aqueous microreactors in a water-in-oil emulsion such that a plurality of aqueous microreactors comprise a single copy of a fragmented nucleic acid, a single bead capable of binding to the fragmented nucleic acid, and amplification reaction solution containing reagents necessary to perform nucleic acid amplification".

See provisional application 60/465,071, paragraph spanning pages 46-47:

> A second approach to amplifying and capturing both strands will be to amplify the fragment library offline in a single tube using oil and surfactant-based emulsions to encapsulate the capture beads, template and PCR reaction mix. This approach will maintain the clonality of the amplification, provide a single-tube format for second strand removal, sequencing primer annealing and the addition of signal-producing enzymes. The average size of the emulsion capsules must be optimized to maximize the number of single beads containing single strands of DNA, that can be incorporated within a single emulsion volume. An adequate volume-to-bead ratio must be maintained in order to insure a maximum number single bead capsules.

amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads, wherein the reagent beads are bound to a plurality of molecules of a primer for amplifying the analyte DNA molecules, whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule

See Leamon claim 1, step c: "amplifying the fragmented nucleic acids in the microreactors to form amplified copies of said nucleic acids and binding the amplified copies to beads in the microreactors".

See Leamon, figures 35-37 and column 7, lines 14-22.

See provisional application 60/465,071, paragraph spanning pages 46-47, quoted above.

See also provisional application 60/465,071, paragraph spanning pages 43-44:

> Each bead is covalently loaded with large numbers of two oligonucleotides complementary to the 3' ends of our two universal linker sequences found on each strand of the amplified product. We will specifically capture (Figure 4) the single stranded forms of the complementary strands of an amplified DNA fragment by using these two oligonucleotides. These oligonucleotides are each targeting the 3' end of their respective stands. Both capture oligonucleotides are conjugated to the solid phase capture bead resulting in the simultaneous capture of the many copies of each strand on the same single bead in a single well.

Note that although in this paragraph, the amplification was discussed as being performed in a sealed well, Leamon's disclosure of the "second approach to amplifying and capturing both strands" (paragraph spanning pages 46-47, quoted above) clearly conveys the contemplation of performing the same amplification in an emulsion.

separating the product beads from analyte DNA molecules which are not bound to product beads

See Leamon figure 37B: "2nd strand removal".

See provisional application 60/465,071, paragraph spanning pages 46-47, quoted above: "...provide a single-tube format for second strand removal...".

determining relative or absolute amounts of product beads comprising one or more sequence features

See Leamon, claim 1, steps d and e: "...delivering the beads to an array of at least 10,000 reaction chambers...performing a sequencing reaction simultaneously on a plurality of the reaction chambers."

See provisional application 60/465,071, page 52, mid-page:

> Since we do not use robotics, but rather count on a Poisson distribution approach for bead deposition, and since we need some practical area for holding the PicoTiterPlate™, we currently only use 272K wells out of 800K wells on a 30 X 60 mm PicoTiterPlate™, to perform sequencing.

See also provisional application 60/465,071, page 2:

> The 454 Corporation has developed a massively parallel, high-throughput sequencing instrument that combines simultaneous sequencing in *hundreds of thousands* of picoliter-scale reaction wells, with high-powered bioinformatics.

Leamon did not teach determining amounts of product beads comprising a first species of analyte DNA molecule as a fraction of product beads (as recited in claim 44) or determining the proportion (i.e. ratio) of product beads comprising a first species of analyte DNA molecule to product beads comprising a second species of analyte DNA molecule (as recited in claim 85). Leamon did not teach that a first species or second species of analyte DNA molecule was wild-type or mutant as recited among claims 86-90.

Vogelstein taught a method to provide "a reliable and quantitative measure of the proportion of variant sequences within a DNA sample" (see abstract). The method provided "a digital readout of the fraction of mutant alleles in the analyzed population" (page 9236, column 2, first paragraph). The method involved isolation of single molecules by dilution, followed by amplification of the single molecules by PCR (see abstract, figure 1). Vogelstein taught (page 9236, column 2, first paragraph) "[t]he

homogeneity of these PCR products makes them easy to distinguish with existing techniques. Such separate amplifications are only useful in a practical sense, however, if a large number of them can be assessed simply and reliably."

In a particular working embodiment, Vogelstein taught (page 9239, first paragraph): "In the case of the tumor with the Gly13Asp mutation, 54% of the positive wells had RED/GREEN ratios >3.0, whereas the other positive wells yielded ratios ranging from 0.9 to 1.1. The PCR products from 16 positive wells were used as sequencing templates (Fig. 4). All the wells yielding a ratio in excess of 3.0 were found to contain mutant *c-Ki-Ras* fragments of the expected sequence, whereas WT sequence was found in the other PCR products." That is Vogelstein determined that of the total products, 54% were mutant, whereas the rest (46%) were wild-type. The difference is that Vogelstein obtained individual molecules for amplification by dilution and dispensing into separate wells of a microplate, whereas Leamon obtained individual molecules for amplification by forming microemulsions. In either case, the amplification products from each amplification were individually sequenced. Vogelstein clearly taught determining the proportion of a first species of DNA (e.g. mutant) as a fraction (54%) of total product, and implicitly taught the proportion of the first species (mutant) to the second species (WT): that being 54% mutant and 46% WT. Moreover, with regard to claims 86-90, it is noted that either the WT or the mutant in Vogelstein's example can be considered as the "first species" or the "second species"; the designation of "first" and "second" in the claim language is not distinguishing.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the high-throughput sequencing technique taught by Leamon to the digital PCR approach of Vogelstein for the purpose of "providing a digital readout of the fraction of mutant alleles in the analyzed population" (Vogelstein, page 9236, column 2, first paragraph). As stated by Vogelstein, "separate amplifications are only useful in a practical sense, however, if a large number of them can be assessed simply and reliably" (page 9236, column 2, first paragraph). In the working example discussed above, Vogelstein sequenced PCR products from only 16 wells (page 9239, column 1, first paragraph). Vogelstein stated (page 9239, second paragraph of "Discussion"): "Dig-PCR [digital PCR] can be used to detect mutations present at relatively low levels in the samples to be analyzed. The limit of detection is defined by the number of wells that can be analyzed and the intrinsic mutation rate of the polymerase used for amplification. The 38-well PCR plates are commercially available, and the 1,536-well plates are on the horizon, theoretically allowing sensitivities for mutation detection at the ~0.1% level." Leamon's method for "digital PCR" (forming microemulsions) and subsequent sequencing of the amplification products using the PicoTiterPlate™ would have allowed the analysis of hundreds of thousands of reactions. One of ordinary skill in the art would clearly have understood the advantage in this, and would have been quite able to adapt Leamon's emulsion PCR/bead capture approach to analyzing the fraction of mutant vs wild-type alleles as disclosed by Vogelstein. What would not have been obvious was the use of flow cytometry, or the use of labeled hybridization probes, as recited in certain claims not

subject to this rejection, since these aspects were clearly not combinable with Leamon's method and indeed would have changed the entire operating principle of Leamon's method.

Conclusion

Claims 37, 39, 43, 60 and 62 are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL C. WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Samuel Woolwine/

Primary Examiner, Art Unit 1637